LA English
OS WPI: 2002-666902 [71]

L7 ANSWER 6 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB DERWENT ABSTRACT:
NOVELTY - A nucleic acid (I) present in other than its natural environment and encoding an Stichodactylidaen **chromoprotein** or its fluorescent mutant, where the fluorescent protein has an emission maximum ranging from 580-660 nm, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a nucleic acid (II) having a sequence of residues that is substantially the same as or identical to a nucleotide sequence of at least 10 residues in length of a sequence (S) of 910, 908, 684, 681 or 687 base pairs given in the specification; (2) a fragment (III) of (I) or (II); (3) an isolated nucleic acid or its mimetic that hybridizes under stringent conditions to (I), (II) or their complementary sequences; (4) a construct comprising a vector and (I), (II), (III), or a nucleic acid or its complement that hybridizes under stringent conditions to the above nucleic acids; (5) an expression cassette (IV) comprising (I), (II), (III), or the above construct, and transcriptional initiation and termination region functional in an expression host; (6) a cell (V) or its progeny comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell; (7) a protein (VI) or its fragment encoded by (I), (II) or (III); (8) an antibody (VII) binding specifically to (VI); (9) a transgenic cell or its progeny comprising (I), (II) or (III); (10) a transgenic organism comprising (I), (II) or (III); and (11) a kit comprising (I), (II) or (III) and instructions for using the nucleic acid.

WIDER DISCLOSURE - Also disclosed are: (1) homologs of (I); (2) nucleic acids that encode proteins encoded by (I), but differ in sequence due to degeneracy of the genetic code; and (3) nucleic acids that encode fusion proteins comprising (VI) fused to a second protein.

BIOTECHNOLOGY - Preparation: (VI) is produced recombinantly. Preferred Nucleic Acid: (I) is isolated. (II) as a sequence similarity of 60% with sequence of 10 residues in (S).

USE - (I), (II) or (III), and (VI) are useful in applications employing a chromo or fluorescent nucleic acid or protein. (V) is useful for producing an **Anthozoan** chromo and/or fluorescent protein (claimed). (III) is useful as primers for polymerase chain reaction (PCR) and hybridization screening probes. (I) is useful to identify expression of the gene in a biological specimen, and to generate transgenic, non-human plants or animals or site specific gene modifications in cell lines. The **chromoproteins**, and their fluorescent mutants are useful as coloring agents capable of imparting color or pigment to a particular composition of matter. The **chromoproteins** can be incorporated into a variety of different compositions including food compositions, pharmaceuticals, cosmetics, living organisms, e.g. animals and plants, and as labels in analyte detection assays, e.g. assays for biological analytes of interest. The **chromoproteins** may be incorporated into adducts with analyte specific antibodies or their binding fragments and subsequently employed in immunoassays for analytes of interest in a complex sample. They are also useful as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms, in sunscreens, as selective filters, and in fluorescence resonance energy transfer (FRET) applications, where the proteins serve as donor and/or acceptors in combination with a second fluorescent protein or dye, e.g. a fluorescent protein. The proteins also

find use as biosensors in prokaryotic and eukaryotic cells, e.g. as Ca2+ ion indicator, as pH indicator, as phosphorylation indicator, as an indicator of other ions, e.g. magnesium, sodium, potassium, chloride and halides and in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis. Screening can be used for drug discovery and in the field of functional genomics e.g. where the subject proteins are used as markers of whole cells to detect changes in multicellular reorganization and migration, e.g. formation of multicellular tubules by endothelial cells, migration of cells, wound healing and neurite outgrowth. The fluorescent protein also finds use in high throughput screening assays, in fluorescence activated cell sorting applications, as a label to mark a population of cells, as in vivo marker in animals, in protease cleavage assays, in assays to determine the phospholipid composition in biological membranes and as a fluorescent timer, in which the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of the fluorescent protein is used to determine the activation/deactivation of gene expression, e.g. developmental gene expression, cell cycle dependent gene expression or circadian rhythm specific gene expression. (VII) is useful for differentiating the fluorescent protein from other fluorescent proteins.

EXAMPLE - A mutant **chromoprotein** C148S was generated. Upon alignment of the **chromoprotein** of 227 amino acids fully defined in the specification with green fluorescent protein (GFP), residue 148 (numbering based on GFP) was identified as being occupied by a Cys residue instead of a Ser residue, where Ser 148 was present in all of the fluorescent Anthozoa derived proteins. Site directed mutagenesis was employed to generate point mutants of the **chromoprotein** containing Ser at position 148. Mutagenesis was performed by the overlap extension method. Two overlapping fragments of each FP coding region were amplified. Forward cloning (5'-acatggatccgctggtttgttgaaaga) and reverse mutagenesis (5'-acctcagtgcttggctcccat) primers were used for 5'-end fragment amplification, and forward mutagenesis (5'-atgggagccaagcactgaggt) and reverse cloning (5'-tgacaagcttctggtgtcactgggaacaatca) primers were used for 3'-end fragment amplification. Polymerase chain reaction (PCR) was carried out using 100 microM of each dNTP, 0.2 microM of each primer and 1 ng of plasmid DNA. To remove plasmids encoding wild type proteins, the 5'- and 3'-fragments were excised. Then 5'-and 3'-fragments were combined to obtain full-length cDNA. The reaction was diluted 10 fold and 1 microl of the diluted sample was used as a template for PCR with forward and reverse cloning primers. Ready-for-cloning fragment containing full-length coding regions with target substitution was generated. This single substitution dramatically increased the quantum yield of red fluorescence as compared to the wild type protein. By random mutagenesis of the primary fluorescent mutant (with Ser 148), a brighter mutant, i.e., 44-9 (hcFRFP) (HcRed), was generated. (81 pages)

AN 2002-16017 BIOTECHDS

TI Novel nucleic acid encoding Stichodactylidaen **chromoprotein** and its fluorescent mutant useful as coloring agent, labels in analyte detection assays, markers in recombinant DNA applications and filters in sunscreens;
vector-mediated recombinant protein gene transfer and expression in host cell, antibody and transgenic animal model construction for use in food, pharmaceutical and cosmetic industries

AU LUKYANOV S A; FRADKOV A F; LUKYANOV K A; GURSKAYA N G

PA CLONTECH LAB INC

PI WO 2002030965 18 Apr 2002

AI WO 2000-US32080 12 Oct 2000

11/08/200413:06Print selected from Online session

PRAI US 2001-306131 16 Jul 2001
DT Patent
LA English
OS WPI: 2002-444170 [47]

L7 ANSWER 7 OF 7 TOXCENTER COPYRIGHT 2004 ACS on STN
AB Kindling fluorescent protein (KFP) compns. and nucleic acids encoding the same, as well as methods for using the same, are provided. In particular, protein FP595 from Anthozoa (also called AsFP595, or FP7, or KFP04) and its two mutants (A148G, and F90L-A148G-H203Y resp.), and another Heteractis crispa **chromoprotein** FP10 (KFP08) and its four mutants (a:K28M-N165A, b:K28M-N165G, c:G20C-T39A-L126H-C148A-N165G-R176H-L181H-A190V-I203H-P208L-K211E, and d:T39A-C148S-N165S-L181H-1203H-P208R-K211E resp.) are provided. These wild-type or mutant kindling fluorescent proteins are expressed recombinantly (as his6 epitope tagged fusion proteins) and purified for further characterization. In general, they become brightly fluorescent proteins, from an initial non-fluorescent or low fluorescent state, upon exposure to a kindling stimulus, which fluorescent state may be reversible or irreversible. Specifically, their kindling wavelength of said kindling stimulus ranges from about 200 to 1500 nm, their kindling stimulus ranges from about 0.01 to about 106 W/cm2, and their kindling duration of said kindling stimulus ranges from about 1 ms to about 60 min. The subject protein/nucleic acid compns. find use in labeling protocols, e.g., in labeling proteins, organelles, cells and organisms, etc., in a variety of different types of applications. Also provided are systems and kits for use in practicing such applications. The use of a KFP to study cell migration during embryogenesis, and to study migration of a mitochondrion are demonstrated.
AN 2002:663560 TOXCENTER
CP Copyright 2004 ACS
DN CA13803021184W
TI Kindling fluorescent proteins from Anthozoa and Heteractis crispa and their mutants and methods for their use
AU Lukyanov, Sergey Anatolievich; Chudakov, Dmitry; Lukyanov, Konstantin
CS ASSIGNEE: Clontech Laboratories, Inc.
PI WO 2002096924 A1 5 Dec 2002
SO (2002) PCT Int. Appl., 96 pp.
CODEN: PIXXD2.
CY UNITED STATES
DT Patent
FS CAPLUS
OS CAPLUS 2002:927445
LA English
ED Entered STN: 20021224
Last Updated on STN: 20040622

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FILE COVERS 1907 - 11 Aug 2004 VOL 141 ISS 7
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This file contains CAS Registry Numbers for easy and accurate substance identification.

=> S WO 2000-US32080/PN,APPS

	0	WO 2000-US32080/PN
	1	WO2000-US32080/AP
	1	WO2000-US32080/PRN
	1	WO 2000-US32080/APPS (WO2000-US32080/AP,PRN)
L8	1	WO 2000-US32080/PN,APPS

=> FILE INPADOC

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6 AUG 2004 <20040806/UPLS>
MOST RECENT INPADOC WEEK: 200432 <200432/EW>
FILE COVERS 1968 TO DATE.

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SIMULTANEOUS LEFT AND RIGHT TRUNCATION (SLART) <<<

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=> TRA L8 PN

L9 TRANSFER L8 1- PN : 4 TERMS
L10 3 L9

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=> S L10 AND US/PC

3939022 US/PC
L11 1 L10 AND US/PC

=> SEL PN

E1 THROUGH E2 ASSIGNED

=> S L10 AND ZA/PC

195120 ZA/PC
L12 0 L10 AND ZA/PC

=> SEL PN

L12 HAS NO ANSWERS

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      2314001 EN/LA
L13           1 L10 AND EP/PC AND EN/LA

=>  SEL PN

E3 THROUGH E3 ASSIGNED

=>  S L10 AND WO/PC AND EN/LA

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L14           1 L10 AND WO/PC AND EN/LA

=>  SEL PN

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      2314001 EN/LA
L15           0 L10 AND CA/PC AND EN/LA

=>  SEL PN

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E1            1     US2002034530/PN
E2            1     US6682754/PN
E3            1     EP1233759/PN
E4            2     WO2001037810/PN


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FILE LAST UPDATED: 10 Aug 2004 (20040810/ED)

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=> SEA E1-E4

	1	US2002034530/PN
	1	US6682754/PN
	1	EP1233759/PN
	1	WO2001037810/PN
		(WO2001037810/PN)
L16	1	(US2002034530/PN OR US6682754/PN OR EP1233759/PN OR WO2001037810/PN)

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L16 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2001:396646 CAPLUS
DN 134:365704
TI In ovo delivery of an immunogen containing implant
IN Emery, Daryll A.; Straub, Darren E.
PA Willmar Poultry Company, Inc., USA

SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001037810	A2	20010531	WO 2000-US32080	20001121 <--
WO 2001037810	A3	20011122		
W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2002034530	A1	20020321	US 1999-449271	19991124 <--
US 6682754	B2	20040127		
EP 1233759	A2	20020828	EP 2000-980673	20001121 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
PRAI US 1999-449271	A	19991124		
WO 2000-US32080	W	20001121		

AB The disclosure provides a method for administering an agent to an avian species by in ovo delivery of an implant releasably containing the agent. In one embodiment, the method is particularly advantageous for stimulating an immune response in a bird by in ovo administration of a biocompatible implant releasably containing an immunogen. The implant can provide for sustained or delayed release of the immunogen or both. The amount of immunogen that is released from the implant into the bird is preferably sufficient to effectively stimulate a primary immune response to the immunogen. Other agents which can be administered according to the method of the invention are disclosed.

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FULL ESTIMATED COST	10.78	104.37

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CA SUBSCRIBER PRICE	-0.74	-3.68

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